

WHAT IS CLAIMED IS:

1 1. A method for identifying a therapeutic agent for use in treating a
2 CAR-mediated disorder or condition, the method comprising:
3 identifying a candidate therapeutic agent by screening one or more
4 compounds to determine whether said compounds can modulate a CAR-mediated
5 intermolecular interaction;
6 administering the candidate therapeutic agent to a test mammal; and
7 determining whether the level of a cholesterol indicator is modulated in
8 said test mammal.

1 2. The method of claim 1, wherein said candidate therapeutic agent is
2 5 β -pregnan-3,20-dione.

1 3. The method of claim 1, wherein said CAR-mediated disorder or
2 condition is selected from the group consisting of: hypercholesterolemia, lipid disorders,
3 atherosclerosis, and cardiovascular disorders.

1 4. The method of claim 1, wherein the mammal is a cholesterol-
2 elevated mammal.

1 5. The method of claim 4, wherein the test mammal has a disruption
2 in both CAR alleles.

1 6. The method of claim 1, wherein said cholesterol indicator is the
2 level of serum cholesterol.

1 7. The method of claim 1, wherein said cholesterol indicator is the
2 level of a member selected from the group consisting of HDL cholesterol, LDL
3 cholesterol, and VLDL cholesterol.

1 8. The method of claim 1, wherein said cholesterol indicator is the
2 mRNA level of a gene involved in the regulation of cholesterol levels.

1 9. The method of claim 1, wherein said CAR-mediated intermolecular
2 interaction is CAR-mediated gene expression.

1 **10.** The method of claim 9, wherein the ability of said candidate
2 therapeutic agent to modulate CAR-mediated gene expression is assessed by:
3 providing a cell that comprises:
4 a) a polynucleotide encoding a fusion polypeptide that
5 comprises: 1) an amino acid sequence that comprises a DNA
6 binding domain of a polypeptide; and 2) a ligand binding
7 domain that is substantially identical to a ligand binding
8 domain of CAR; and
9 b) a reporter gene construct which comprises a response element
10 to which the DNA binding domain can bind, wherein the
11 response element is operably linked to a promoter that is
12 operative in the cell and the promoter is operably linked to a
13 reporter gene; and
14 contacting said cell with said candidate therapeutic agent; and
15 determining whether said reporter gene is expressed at a higher or lower
16 level in the presence of said candidate therapeutic agent as compared to expression in the
17 absence of said candidate therapeutic agent.

1 **11.** The method of claim 10, wherein said candidate therapeutic agent
2 is 5 β -pregnan-3,20-dione.

1 **12.** The method of claim 10, wherein said DNA binding domain is
2 substantially identical to a DNA binding domain from a polypeptide selected from the
3 group consisting of: CAR, a GAL4 transcription factor, an estrogen receptor, a
4 progesterone receptor, a glucocorticoid receptor, an androgen receptor, a mineralcorticoid
5 receptor, a vitamin D receptor, a retinoid receptor, and a thyroid hormone receptor.

1 **13.** The method of claim 12, wherein said DNA binding domain is a
2 CAR DNA binding domain and the response element is a CAR response element.

1 **14.** The method of claim 13, wherein said CAR response element is a
2 DR-5 element or a DR-4 element.

1 **15.** The method of claim **10**, wherein said reporter gene encodes a
2 marker protein selected from the group consisting of: luciferase, alkaline phosphatase,
3 beta-galactosidase, chloramphenicol acetyltransferase and green fluorescent protein.

1 **16.** The method of claim **1**, wherein said CAR-mediated intermolecular
2 interaction is the binding of a polypeptide that comprises a CAR ligand binding domain to
3 a ligand for CAR.

1 **17.** The method of claim **16**, wherein said polypeptide is a CAR α or a
2 CAR β .

1 **18.** The method of claim **16**, wherein said ligand for CAR comprises a
2 sensor peptide.

1 **19.** The method of claim **18**, wherein said ligand for CAR comprises a
2 receptor binding domain of a coactivator or a corepressor.

1 **20.** The method of claim **19**, wherein said coactivator is SRC-1.

1 **21.** The method of claim **20**, wherein said sensor peptide is rhodamine
2 labeled ILRKLLQE.

1 **22.** The method of claim **16**, wherein the binding of the polypeptide
2 that comprises a CAR ligand binding domain to the ligand for CAR is determined in the
3 presence of a naturally occurring ligand for CAR.

1 **23.** The method of claim **22**, wherein said naturally occurring ligand
2 for CAR is 5 β -pregnan-3,20-dione.

1 **24.** The method of claim **16**, wherein said method comprises
2 determining whether said compound can inhibit the interaction between the CAR ligand
3 binding domain and the CAR ligand.

1 **25.** The method of claim **24**, wherein said CAR ligand is labeled.

1 **26.** The method of claim **25**, wherein said CAR ligand is radiolabeled.

1 **27.** The method of claim **24**, wherein said CAR ligand is labeled with a
2 fluorophore.

1 **28.** The method of claim **27**, wherein said method comprises a
2 fluorescence polarization assay.

1 **29.** The method of claim **27**, wherein said method comprises a
2 fluorescence resonance energy transfer assay.

1 **30.** The method of claim **27**, wherein said CAR is labeled with a
2 fluorophore.

1 **31.** The method of claim **30**, wherein said method comprises a
2 fluorescence resonance energy transfer assay or a fluorescence polarization assay.

1 **32.** The method of claim **24**, wherein said CAR ligand is selected from
2 the group consisting of:

3 5 α -androst-16-en-3 α -ol, 5 α -androst-16-en-3 α -ol acetate, 5 α -androstane-
4 3 α -ol, 5 α -androst-16-en-3 α -ol acetate and 5 β -pregnan-3,20-dione.

1 **33.** A method for identifying a therapeutic agent for use in treating a
2 CAR-mediated disorder or condition the method comprising:
3 administering a compound to a CAR compromised mammal; and
4 determining whether administration of the compound results in a change in
5 cholesterol level compared to a mammal to which the compound is not administered.

1 **34.** The method of claim **33**, wherein the method further comprises
2 administering the compound to a CAR non-compromised mammal and comparing the
3 effect on the cholesterol level indicator of administering the compound to that of
4 administering the compound to the CAR compromised mammal.

1 **35.** The method of claim **33**, wherein said cholesterol level indicator is
2 the level of serum cholesterol.

1 **36.** The method of claim **33**, wherein said cholesterol level indicator is
2 the level of a member selected from the group consisting of HDL cholesterol, LDL
3 cholesterol, and VLDL cholesterol.

1 **37.** The method of claim **33**, wherein said cholesterol level indicator is
2 the mRNA level of a gene involved in the regulation of cholesterol levels.

1 **38.** The method of claim **33**, wherein said CAR compromised mammal
2 is a mammal having a disruption in both CAR alleles.

1 **39.** The method of claim **38**, wherein said CAR compromised mammal
2 is a mouse.

1 **40.** The method of claim **38**, wherein said disruption occurs in the
2 coding region for the DNA binding domain of CAR.

1 **41.** The method of claim **38**, wherein said disruption in a CAR allele
2 comprises an insertion at codons for amino acid positions from about amino acid 21 to
3 about amino acid 86 of CAR β .

1 **42.** A method for treating a CAR-mediated disorder or condition, the
2 method comprising:
3 administering to a mammal having a CAR-mediated disorder or condition
4 an effective amount of a therapeutic agent that modulates CAR-mediated regulation of
5 cholesterol levels.

1 **43.** The method of claim **42**, wherein said therapeutic agent is
2 identified by:
3 screening one or more compounds to determine whether said compounds
4 can modulate a CAR-mediated intermolecular interaction;
5 administering the candidate therapeutic agent to a test mammal; and
6 determining whether the level of a cholesterol indicator is affected in said
7 test mammal.

1 **44.** The method of claim **42**, wherein said CAR-mediated disorder or
2 condition is selected from the group consisting of: hypercholesterolemia, lipid disorders,
3 atherosclerosis, and cardiovascular disorders.

1 **45.** A non-human mammal having a genome that comprises a
2 disruption in at least one CAR allele.

1 **46.** The non-human mammal of claim **45**, wherein said disruption
2 comprises an insertion, deletion or mutation in a region of the CAR allele that encodes for
3 a DNA binding domain of CAR.

1 **47.** The non-human mammal of claim **46**, wherein said disruption
2 comprises an insertion at codons for amino acid positions from 21 to about 86 of CAR β .

1 **48.** A non-human mammal having a genome that comprises a
2 disruption in both CAR alleles.

1 **49.** The non-human mammal of claim **48**, wherein said disruption
2 comprises an insertion, deletion or mutation in a region of the CAR allele that encodes for
3 a DNA binding domain of CAR.

1 **50.** The non-human mammal of claim **48**, wherein said disruption
2 comprises an insertion at codons for amino acid positions from 21 to about 86 of CAR β .

1 **51.** The non-human mammal of claim **48**, wherein said non-human
2 mammal exhibits an increased level of serum cholesterol relative to a wild-type mammal.

1 **52.** A method for producing a transgenic non-human mammal having a
2 genome that comprises a disrupted CAR allele, the method comprising:
3 introducing into embryonic stem cells a polynucleotide that comprises a
4 coding region for a portion of a CAR polypeptide, wherein the polynucleotide sequence
5 includes a disruption in the coding region of a portion of said CAR polypeptide;
6 identifying a cell into which said polynucleotide sequence has been
7 integrated into an endogenous CAR allele;

8 introducing said cell into a blastocyst, thereby forming a transgenic
9 blastocyst;
10 implanting said transgenic blastocyst into a pseudopregnant mammal and
11 allowing said pseudopregnant mammal give birth to a transgenic mammal.

1 **53.** The method of claim **52**, wherein said transgenic mammal contains
2 a disrupted CAR allele in its germline.

1 **54.** The method of claim **53**, further comprising breeding said
2 transgenic mammal to generate a heterozygous mammal comprising a disrupted CAR
3 allele.

1 **55.** The method of claim **53**, further comprising mating a male and a
2 female mammal each heterozygous for said disrupted CAR allele, to form progeny that
3 are homozygous for said disrupted CAR allele.

1 **56.** The method of claim **52**, wherein said disrupted CAR allele
2 comprises an insertion into a region of the CAR allele that codes for a DNA binding
3 domain of CAR.

1 **57.** The method of claim **52**, wherein said disrupted CAR allele
2 comprises an insertion at codons for amino acid positions from about 21 to about 86 of
3 CAR β .

1 **58.** The method of claim **56**, wherein said insertion comprises a
2 selectable marker gene.

1 **59.** The method of claim **58**, wherein said marker gene encodes for
2 neomycin resistance.